DETERMINATION OF VILDAGLIPTIN USING A SIMPLE AND SENSITIVE FLUORESCENT PROBE**

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Vildagliptin (VLG), a drug for the treatment of type 2 diabetes, is nonfluorescent in aqueous solution. This property makes it difficult to determine by direct fluorometric methods. We proposed a new competitive method for fluorometric detection of VLG using CB[7]–BER (cucurbit[7]uril = CB[7], BER = berberine) as a fluorescent probe. The method showed a good calibration curve within the concentration range 0.00213–1.820 μg/mL with an excellent correlation coefficient (r^2>0.999). The detection limit is 0.64 ng/mL for CB[7]–BER fluorescent probe. Moreover, the method was successfully applied for the determination of VLG in pharmaceutical tablets and artificial urine. To our knowledge, this is the first example of determining VLG using a fluorescent probe method.

Keywords: *vildagliptin, berberine, cucurbituril, fluorescent probe.*

ОПРЕДЕЛЕНИЕ ВИЛДАГЛИПТИНА С ПОМОЩЬЮ ПРОСТОГО И ЧУВСТВИТЕЛЬНОГО ФЛУОРЕСЦЕНТНОГО ЗОНДА

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Вилдаглиптин (VLG), препарат для лечения диабета второго типа, не флуоресцирует в водном растворе, что затрудняет его определение прямыми флуориметрическими методами. Предложен конкурентный метод флуориметрического обнаружения VLG с использованием CB[7]–BER (кукурбит[7]урил = CB[7], BER = берберин) в качестве флуоресцентного зонда. Показана хорошая корреляция метода в диапазоне концентраций 0.00213–1.820 мкг/мл с коэффициентом r^2 > 0.999. Для флуоресцентного зонда CB[7]–BER предел обнаружения 0.64 нг/мл. Метод успешно применен для определения VLG в фармацевтических препаратах и синтетической урине.

Ключевые слова: *вилдаглиптин, берберин, кукурбитурил, флуоресцентный зонд.*

Introduction. Glucagon-like peptide 1 (GLP-1) is an important hormone that can maintain glucose concentration in vivo and has a incretin effect [1, 2]. GLP-1 can promote islet hormone secretion in patients with type 2 diabetes, which acts on the insulin beta cells [3]. Dipeptidyl peptidase IV (DPP4) is a proteolytic

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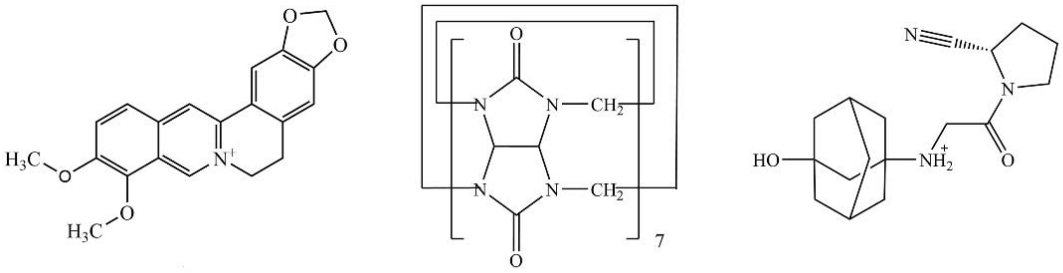
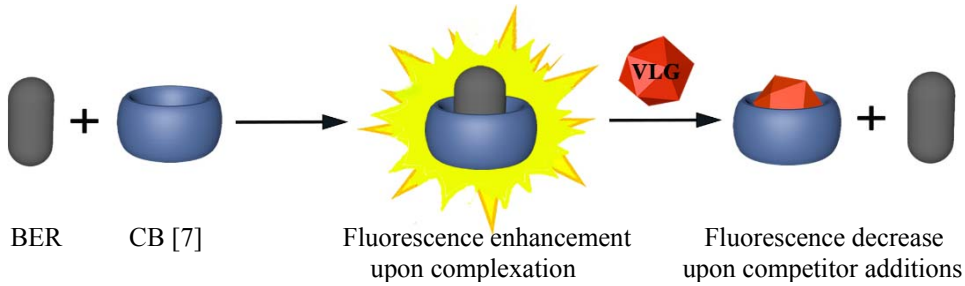
enzyme widely distributed in various human organs and tissues, and it can quickly inactivate GLP-1. Vildagliptin (VLG), (s)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyr-rrolidine-2-carbonitrile (Scheme 1) is a selective, competitive, and reversible DPP4 inhibitor for the treatment of type 2 diabetes [4–7]. It has a significant role in lowering blood glucose by inhibiting the activity of DPP4 and increasing the concentration of GLP-1, and has no effect on weight [8, 9]. Many methods are used to analyze VLG in drug detection applications, such as ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QTOF-MS) [10], liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF/MS) [11], capillary zone electrophoresis (CZE) [12], high-performance thin-layer chromatography (HPTLC) [1], voltammetric determination [13], and ultraperformance liquid chromatography method with tandem mass detection [14]. However, HPLC generally requires expensive equipment, complicated apparatuses, labor-intensive sample preparation procedures, and personnel skills in chromatographic techniques, but other methods are not sensitive enough. Therefore, a simple, rapid, highly sensitive, and selective method, such as spectrofluorimetry is needed for the determination of VLG. To the best of our knowledge, no fluorometric method is available for the detection of VLG.

Cucurbit[*n*] urils (CB[*n*]s, *n* = 5–8, 10) are a family of pumpkin-shaped macrocyclic molecules composed of *n* glycoluril units connected with 2*n* methylene groups [15–17]. As highly symmetrical host molecules, CB[*n*]s was composed of a rigid hydrophobic cavity and two identical polar portals landed with ureido carbonyl groups, making them excellent binders of cationic organic guests [18–24]. Among different CB[*n*]s, CB[7] (Scheme 1) is the most widely used host molecule owing to its good water solubility and the formation of stable inclusion complexes [25–27]. Recently, CB[7]-based host–guest complexes have been generally utilized in some application fields, such as drug detection [28], optical sensors [29], drug delivery [30], biomimetic catalysis [31], and molecular switch [32].



Scheme 1. Structure of BER, CB[7], and VLG.

Berberine (BER) is a natural isoquinoline alkaloid that exhibits weak native fluorescence in aqueous solution [33]. However, in preliminary studies, it was observed that the fluorescence of BER in aqueous solutions was significantly increased in the presence of CB[7] [29, 34]. In this work, we employed CB[7]–BER as a fluorescent probe to detect the nonfluorescent VLG (Scheme 2), which is based on the competition between the VLG and BER for the hydrophobic cavity of CB[7]. To our knowledge, the use of CB[7]–BER as a fluorescent probe for the determination of VLG has not yet been reported. The proposed method is more sensitive than any other methods reported in the literature [35–38], and was used for the determination of VLG in pharmaceutical tablet and artificial urine samples, which shows promising application in various practical scenarios.



Scheme 2. Competitive recognition of CB[7] to VLG against BER.

Experimental. BER and VLG were obtained from the Chinese National Institute for the Control of Pharmaceuticals. CB[7] was prepared and characterized according to the literature [39]. Stock solutions of BER, VLG, and CB[7] (100 μM) were dissolved in fresh double-distilled water. All the standard stock solutions can be stored for several weeks at room temperature. Standard working solutions were obtained by dilution of the stock solutions with fresh double-distilled water before use. All other reagents used were of analytical reagent grade.

The fluorescence spectroscopy was measured by an Edinburgh FLS55 spectrophotometer. The slit widths of both excitation and emission monochromators were set to 5 nm. UV-Visible spectroscopy was performed on a Shimadzu UV-2600 UV-Visible spectrophotometer. All measurements were performed using a standard 1-cm path-length quartz cell at room temperature. The ^{1}H NMR spectra were conducted using a Bruker AV-400 MHz NMR spectrometer in D_2O.

The fluorescence spectra and intensity measurements were carried out with the excitation wavelength of 350 nm in the absence or presence of VLG solution. In a 10-mL colorimetrical cylinder, 1 mL (0.01 mM) CB[7] solution, 1 mL (0.01 mM) BER solution, followed by 1.0 mL (0.01 M) hydrochloric acid, and a given concentration of VLG standard solution or sample solution were sequentially added. The mixture was diluted to the volume with double-distilled water and shaken for 20 min at room temperature.

The contents of 10 tablets of a VLG drug were carefully powdered. A portion of this powder equivalent to 10 mg VLG was accurately weighed, dissolved with double-distilled water in a 100-mL volumetric flask, and sonicated for 20 min. The solution was then diluted to the indicated volume line with double-distilled water. The first 10 mL of the filtrate was discarded, after which 10 mL of the remaining filtered sample solution was diluted to 100 times its volume with double-distilled water. Further dilutions were made to obtain sample solutions using the same detection methods.

Urine was obtained from commercial artificial urine, which was produced in Beijing Solarbio Science & Technology Co., Ltd. For urine analysis, urine was diluted and its analyte comprised 10% artificial urine. The as-obtained urine samples were next spiked with different levels of VLG. Finally, the samples were treated with probe CB[7]–BER solution (10 μM), and fluorescence was measured.

Results and discussion. *The fluorescence quenching of CB[7]–BER by VLG.* Although the fluorescent emission of pure BER was very weak or undetectable, the fluorescence intensity was greatly enhanced after it entered the hydrophobic cavity of CB[7], and it was confirmed to form 1:1 host–guest stable complex between CB [7] and BER [40, 41]. The apparent association constant of the complex was 1.77×10^5 M^{-1} [41].

When gradually adding VLG to the CB[7]–BER solution, the fluorescence intensity of CB[7]–BER rapidly decreased (Fig. 1a), which is presumably due to the competitive reaction between VLG and BER for the occupancy of the CB[7] cavity. Parts of the BER molecules were expelled from CB[7] cavities by the introduction of VLG, thereby reducing the fluorescence intensity of CB[7]–BER owing to the formation of a new inclusion complex CB[7]–VLG. The hyperchromic effect was also observed after the addition of VLG to CB[7]–BER solution (Fig. 2). The binding constant of CB[7]–VLG was extremely large 1.97×10^9 M^{-1} (Fig. 1b), indicating very strong host–guest interactions with excellent size and shape matches. The fluorescence intensity decrease to the amount of added VLG led to a simple and sensitive fluorescence method for the determination of VLG.

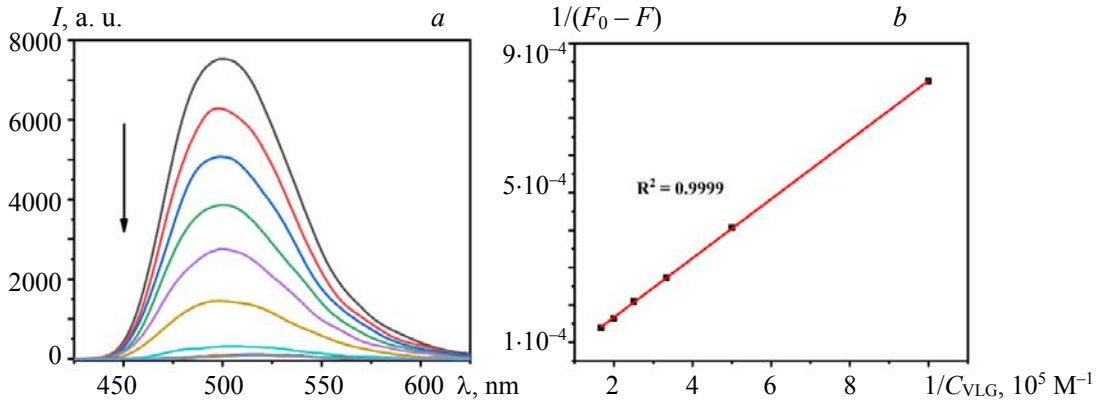


Fig. 1. Fluorescence spectra of CB[7]–BER in different concentrations of VLG from 0 to 10 μM (a); the binding constant for the CB[7] with VLG ascertained from the fluorescence titration (b), (pH 5.0, λ_{ex} = 350 nm).

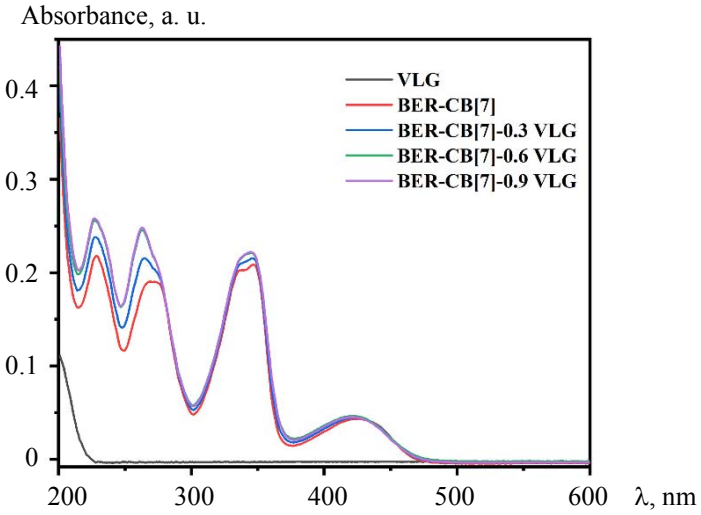


Fig. 2. The UV-visible spectra of CB[7]–BER (10 μM) in the absence and presence of increasing concentration of VLG (0–9 μM).

Optimization of experimental conditions. The effect of pH within the range 3.0 to 11.0 was investigated. The result indicated that the fluorescence-quenching values (ΔF) changed slightly within the pH range 3.0 to 7.0, and displayed the maximum change at pH 5.0. However, at pH > 7.0, ΔF decreased relatively more significantly, possibly due to alkali cations were readily coordinated with the carbonyl groups of CB[7], and the rate constant of the ingress of the organic guest ingression was lower at a higher pH [42].

Hence, using hydrochloric acid, the pH was adjusted to 5.0, which was the desired pH for all subsequent experiments. In addition, ΔF reached a maximum 20 min after the reagents were added and it remained constant for at least 12 h. Hence, the standard reaction condition was set to pH 5.0 for 20 min.

The response mechanism of the host–guest inclusion complex. BER exhibits weak fluorescence in aqueous solution because the isoquinoline ring and the substituted benzene ring in BER is not in the same plane. Thus, the BER molecule cannot form a conjugated system. When CB[7] is added to the aqueous solution of BER, the nonpolar isoquinoline moiety of BER penetrates into the hydrophobic cavity of CB[7], causing electrostatic attraction between the high electron density of the carbonyl oxygens of CB[7] and the positive charge of the heterocyclic nitrogen of BER. Hence, the degree of freedom of motion of BER molecule is reduced, and further reduced the probability of nonradiative transition, which leads to fluorescence enhancement. The formation of the CB[7]–BER complex has been confirmed previously by ^{1}H NMR spectroscopy [40].

When VLG was added to CB[7]–BER solution, VLG could compete to occupy the CB[7] cavity with BER. Owing to the association constant of CB[7]–VLG (1.97×10^9 M^{-1}) was greater than CB[7]–BER (1.77×10^5 M^{-1}); thus, BER in the CB[7] cavity was displaced by VLG. As a result, the fluorescent intensity of the CB[7]–BER complex was weakened. ^{1}H NMR experiments between VLG and CB[7]–BER inclusion complexes were recorded to investigate competitive interaction. As shown in Fig. 3, upon addition of VLG to the CB[7]–BER solution, the signals of the protons (H8, H11, H12, H13, H15, and H16) of BER shifted to downfield relative to free BER, and the signals of the H2, H4, H5, H6, H7, H8, H9, and H10 of VLG shifted to upfield relative to free VLG. These findings indicated that the VLG molecules were included in the CB[7] cavity, and BER molecules were expelled from the CB[7] cavity by VLG molecules.

Analytical performance. Under the optimal experimental conditions described, the standard calibration curves were drawn by plotting ΔF versus the concentration of VLG. With increasing concentrations of VLG, the fluorescence intensity of CB[7]–BER decreased gradually, and ΔF exhibited a good linear relationship with VLG within the range 0.00213–1.820 μg/mL. The linear regression equations were $\Delta F = 12824.383C +$ + 263.208 (C represents the concentration in 10^{-5} M) with correlation coefficients of 0.9998 (Fig. 4). The limit of detection (LOD) and the limit of quantification (LOQ) were 0.64 and 2.13 ng/mL for CB[7]–BER (LOD = 3σ/S, LOQ = 10σ/S), where σ and S are the relative standard deviation of the response and the slope of the calibration curve respectively. The method was proved to have higher sensitivity than other detection methods for VLG detection (Table 1) [35–38].

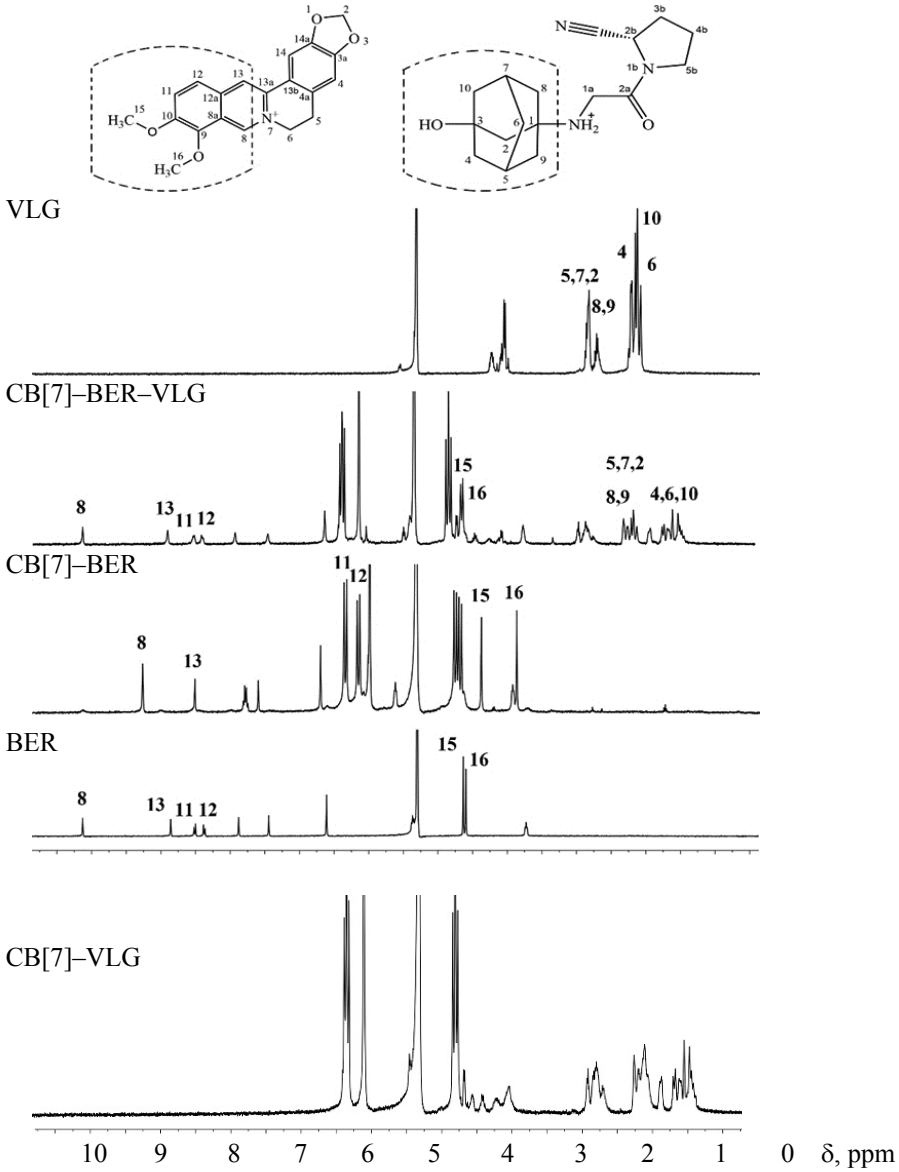


Fig. 3. ^{1}H NMR spectrum BER (9.1 mM), CB[7]–1.0 BER, CB[7]–1.0 BER–1.0 VLG, VLG, and CB[7]–VLG.

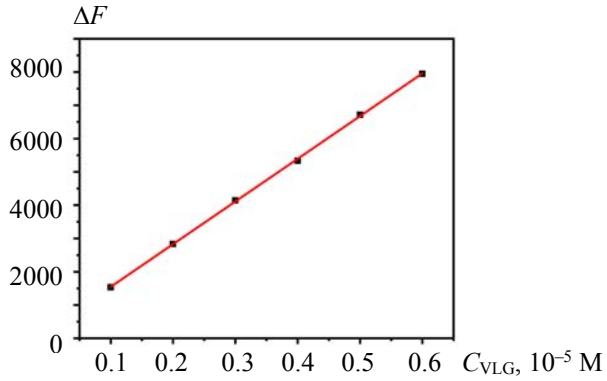


Fig. 4. The standard calibration curve Δ*F* versus VLG, using CB[7]–BER as fluorescence probe.

TABLE 1. Comparison with Other Methods for the Determination of VLG

Technique	Linear range, μg/mL	Detection limit, μg/mL	Ref.
LC-MS/MS	0.02–1.6	0.004	[35]
GC-MS	0.0035–0.3	0.002	[36]
CEZ	30–60	2.820	[37]
RP HPLC	10–100	1.000	[38]
Spectrofluorimetry (CB[7]–BER)	0.00213 to 1.8204	0.00064	This work

Analytical application. The fluorescent method was then applied in the determination of the VLG in pharmaceutical tablets. The analytical results are summarized in Table 2. The relative standard deviations obtained from the proposed method were less than 0.93%. In addition, to confirm the applicability of the method, VLG was added to the tablets and tested by the standard addition method, and the percentage recoveries were 97.00±0.25 to 101.50±0.30, indicating both good accuracy and precision.

TABLE 2. Determination of VLG Pharmaceutical Tablets Using CB[7]–BER (n = 5)

Sample	Content, μg/mL	Spiked, μg/mL	Found, μg/mL	% Spiked recovery ± SD
1	0.4854	0.3034	0.7797	97.00 ± 0.25
2	0.4854	0.6068	1.1013	101.50 ± 0.30
3	0.4854	0.9102	1.3956	100.00 ± 0.04

The method was also applied in the determination of the VLG in spiked samples of artificial urine. The results are presented in Table 3. The relative standard deviations obtained from the fluorescent probe (CB[7]–BER) method were less than 1.10%. The satisfactory percentage recoveries were within the range 98.93±0.21 to 102.30±0.12. These results indicated that the proposed method is effective for the detection of urine samples.

TABLE 3. Determination of VLG in Spiked Urine Using CB[7]–BER (n = 5)

Sample	Added, μg/mL	Found, μg/mL	% Recovery ± SD
Urine1	0.3034	0.3064	101.00 ± 0.10
Urine 2	0.6068	0.6208	102.30 ± 0.12
Urine 3	0.9102	0.9005	98.93 ± 0.21

Conclusions. A highly sensitive and simple fluorometry method was first established for the determination of VLG using the CB[7]–BER complex as a fluorescence probe based on host–guest competitive reaction. The results show that VLG can quench the intensity of the fluorescent probe. The detection limit was 0.64 ng/mL for CB[7]–BER. Moreover, the method has also been successfully applied for the detection of VLG in pharmaceutical tablets and human urine, and satisfactory percentage recoveries within the range 97.00±0.25 to 101.50±0.30 for CB[7]–BER were obtained. These results indicated that the proposed method is effective for the detection of VLG in pharmaceutical tablets and artificial urine samples. The present study is beneficial to molecular recognition between CB[7] and drugs that have nonfluorescent or weakly fluorescent substances.

REFERENCES

1. E. I. El-Kimary, D. A. Hamdy, S. S. Mourad, M. A. Barary, *J. Chromatogr. Sci*., **54**, No. 1, 79–87 (2015).
2. S. Mowaka, D. Mohamed, *RSC Adv*., **74**, No. 5, 60467–60481 (2015).
3. S. Shantikumar, N. Satheeshkumar, B. Prasanth, A. Lingesh, D. Paul, R. Srinivas, *Anal. Methods*, **7**, No. 15, 6198–6206 (2015).
4. M. Attimarad, S. H. Nagaraja, B. E. Aldhubaib, A. Nair, *Diabetes*, **1**, 4 (2014).
5. A. T. Barden, B. Salamon, E. E. S. Schapoval, M. Steppe, *J. Chromatogr. Sci*., **50**, No. 5, 426–432 (2012).
6. T. Boovizhikannan, V. K. Palanirajan, *J. Pharm. Res*., **7**, No. 1, 113–116 (2013).

7. R. Pontarolo, A. C. Gimenez, T. M. G. de Francisco, R. P. Ribeiro, F. L. D. Pontes, J. C. Gasparetto, *J. Chromatogr. B*, **965**, 133–141 (2014).
8. A. T. Barden, B. L. Piccoli, N. M. Volpato, M. Steppe, *Anal. Methods*, **5**, No. 20, 5701–5708 (2013).
9. M. Fadr, A. N. Amro, S. B. Aoun, *Trop. J. Pharm. Res*., **17**, No. 9, 1847–1852 (2018).
10. M. M. Fachi, L. B. Cerqueira, L. P. Leonart, T. M. G. D. Francisco, R. Pontarolo, *PloS One*, **11**, No. 12, e0167107 (2016).
11. S. Shantikumar, N. Satheeshkumar, B. Prasanth, A. Lingesh, D. Paul, R. Srinivas, *Anal. Methods*, **7**, No. 15, 6198–6206 (2015).
12. A. Barden, B. Piccoli, N. Volpato, E. Schapoval, M. Steppe, *Die Pharm. Int. J. Pharm. Sci.*, **69**, No. 2, 86–91 (2014).
13. M. Fadr, A. N. Amro, S. B. Aoun, *Trop. J. Pharm. Res.*, **17**, No. 9, 1847–1852 (2018).
14. R. I. El Bagary, H. M. Azzazy, E. F. El Kady, F. Farouk, J. Liq, *Chromatogr. Related Technol.*, **39**, No. 4, 195–202 (2016).
15. O. V. D. Oliveira, G. D. C. Costa, L. T. Costa, *J. Phys. Chem. B*, **122**, No. 50, 12107–12113 (2018).
16. S. R. Wang, J. Q. Wang, G. H. Xu, L. Wei, B. S. Fu, L. Y. Wu, Y. Y. Song, X. R. Yang, C. Li, S. M. Liu, *Adv. Sci*., **5**, No. 7, 1800231 (2018).
17. S. Li, I. W. Wyman, C. Wang, Y. Wang, D. H. Macartney, R. Wang, *J. Org. Chem*., **81**, No. 19, 9494–9498 (2016).
18. M. Gupta, K. Parvathi, S. Mula, D. K. Maity, A. K. Ray, *Photochem. Photobiol. Sci*., **16**, No. 4, 499–506 (2017).
19. Z. Huang, H. Zhang, H. Bai, Y. Bai, S. Wang, X. Zhang, *ACS Macro Lett*., **5**, No. 10, 1109–1113 (2016).
20. L. Scorsin, J. A. Roehrs, R. R. Campedelli, G. F. Caramori, A. O. Ortolan, R. L. Parreira, H. D. Fiedler, A. Acuna, L. Garcia-Rio, F. Nome, *ACS Catalysis*, **8**, No. 12, 12067–12079 (2018).
21. M. A. Gamal-Eldin, D. H. Macartney, *Org. Biomol. Chem*., **11**, No. 3, 488–495 (2013).
22. J. Robinson-Duggon, F. Perez-Mora, L. Valverde-Vasquez, D. Cortes-Arriagada, J. R. De la Fuente, G. Gunther, D. Fuentealba, *J. Phys. Chem. C*, **121**, No. 39, 21782–21789 (2017).
23. P. Xu, Q. Feng, X. Yang, S. Liu, C. Xu, L. Huang, M. Chen, F. Liang, Y. Cheng, *Bioconjugate Chem*., **29**, No. 8, 2855–2866 (2018).
24. H. S. El-Sheshtawy, S. Chatterjee, K. I. Assaf, M. N. Shinde, W. M. Nau, J. Mohanty, *Sci. Rep.*, **8**, No. 1, 1–10 (2018).
25. S. Senler, B. Cheng, A. E. Kaifer, *Org. Lett*., **16**, No. 22, 5834–5837 (2014).
26. M. H. Tootoonchi, S. Yi, A. E. Kaifer, *J. Am. Chem. Soc*., **135**, No. 29, 10804–10809 (2013).
27. G. Villarroel-Lecourt, J. Carrasco-Carvajal, F. Andrade-Villalobos, F. Solis-Egana, I. Merino-San Martín, J. Robinson-Duggon, D. Fuentealba, *ACS Omega*, **3**, No. 7, 8337–8343 (2018).
28. Y. Li, C.-F. Li, L.-M. Du, J.-X. Feng, H.-L. Liu, Y.-L. Fu, *Talanta*, **132**, 653–657 (2015).
29. H. Bai, H. Chen, R. Hu, M. Li, F. Lv, L. Liu, S. Wang, *ACS Appl. Mater. Interfaces*, **8**, No. 46, 31550–31557 (2016).
30. C. Kim, S. S. Agasti, Z. Zhu, L. Isaacs, V. M. Rotello, *Nature Chem*., **2**, No. 11, 962–966 (2010).
31. A. Palma, M. Artelsmair, G. Wu, X. Lu, S. J. Barrow, N. Uddin, E. Rosta, E. Masson, O. A. Scherman, *Angew. Chemie*, **129**, No. 49, 15894–15898 (2017).
32. Q. Li, Y. Wu, H. Lu, X. Wu, S. Chen, N. Song, Y.-W. Yang, H. Gao, *ACS Appl. Mater. Interfaces*, **9**, No. 11, 10180–10189 (2017).
33. S. Fu, S. Ni, D. Wang, M. Fu, T. Hong, *Int. Immunopharm*., **71**, 1–6 (2019).
34. G.-Q. Wang, L. Guo, L.-M. Du, Y.-L. Fu, *Microchem. J*., **110**, 285–291 (2013).
35. M. Al Bratty, H. A. Alhazmi, S. A. Javed, K. G. Lalitha, M. Asmari, J. Wölker, S. El Deeb, *Chromatographia*, **80**, No. 6, 891–899 (2017).
36. E. Uçaktürk, *J. Anal. Methods in Chem*. (**2015**).
37. A. T. Barden, B. L. Piccoli, N. M. Volpato, M. Steppe, *Anal. Methods*, **5**, No. 20, 5701–5708 (2013).
38. A. Kashid, D. Ghorpade, P. Toranmal, S. Dhawale, *J. Anal. Chem*., **70**, No. 4, 510–515 (2015).
39. J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi, K. Kim, *J. Am. Chem. Soc*., **122**, No. 3, 540–541 (2000).
40. M. Megyesi, L. Biczók, I. Jablonkai, *J. Phys. Chem. C*, **112**, No. 9, 3410–3416 (2008).
41. C. Li, J. Feng, H. Ju, *Analyst*, **140**, No. 1, 230–235 (2015).
42. L. Shi, J.-H. Xie, L.-M. Du, Y.-X. Chang, H. Wu, *Spectrochim. Acta A: Mol. Biomol. Spectr*., **162**, 98–104 (2016).